IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: Larry R. Helms
Claude F. Meares, et al.	Technology Center/Art Unit: 1642
Application No.: 09/671,953	DECLARATION OF CLAUDE MEARES
Filed: September 27, 2000	UNDER 37 C.F.R. § 1.132
For: ENGINEERING ANTIBODIES THAT BIND IRREVERSIBLY	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Claude Meares, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I received my Ph.D. in chemistry from Stanford University in 1972 and my B.S., with highest honors from the University of North Carolina, Chapel Hill in 1968. I am presently a Professor of Chemistry at the University of California, Davis, and the Chief Scientific Advisor for Lexrite Labs, Inc., the licensee of the above-identified patent application. I have been in these and related positions since 1972. I have over 200 scientific publications in the fields of chemistry, nuclear medicine, bioconjugate chemistry and engineered antibodies. A copy of my CV is attached as Exhibit A.

3, I have read and am familiar with the contents of the subject patent application. I have also read the Office Action received from the United States Patent and Trademark Office dated October 28, 2003. It is my understanding that the Examiner is

concerned that (1) the claimed compositions are not novel in view of Stickney *et al.*, *Cancer Res.* 51: 6650 (1991); (2) the claimed compositions are obvious in view of Reardan *et al.*; *Nature* 316:265 (1985); Orlandi *et al., PNAS USA* 86:3833 (1989), Pastan *et al.*, U.S. Patent No. 5,747,654; and Goodwin *et al., J. Nucl. Med.* 29:226 (1988), and (3) the claimed compositions are not enabled by the specification.

4. The present invention is directed to mutant antibodies comprising (1) a reactive site not present in the wild-type of said antibody and (2) complementarity-determining regions (CDRs) that recognize a metal chelate. The reactive site is in a position proximate to or within said complementarity-determining regions. The reactive site results from the mutation and interacts with a complementary reactive group on the metal chelate. The reactive group on the metal chelate is selected from carboxyl groups, hydroxyl groups, haloalkyl groups, dienophile groups, aldehyde groups, ketone groups, sulfonyl halide groups, thiol groups, amine groups, sulfhydryl groups, alkene groups, and epoxide groups. This declaration is presented to demonstrate that the claimed mutant antibodies are novel and nonobvious in view of the cited references and that the claimed compositions are enabled by the specification as filed.

5. *The claimed compositions are novel over the disclosure of Stickney* et al.

The Examiner alleges that Stickney *et al.* describe a mutant antibody in which the (Fab')2 is generated by chemically linking the binding sites of two separate antibodies with a bis-maleimidomethyl ether to create a mutant antibody. In particular, the Examiner alleges that the linker is a mutation that is a reactive site and concludes that the antibody of Stickney *et al.* contains all of the elements of the claimed mutant antibodies.

A mutation is a substitution, addition, or deletion in a nucleotide sequence encoding a polypeptide of interest (*see*, specification at *e.g.*, page 23, lines 26-28). In the case of an antibody, a mutant antibody is generated by introducing a substitution, addition, or deletion into the nucleotide sequence encoding the antibody. The chemically produced antibody described in Stickney *et al.* is not a mutant antibody. As explicitly set forth in Stickney *et al.*, the antibody is formed by ***chemically*** joining a Fab' fragment from a monoclonal antibody that specifically recognizes a carcinoembryonic antigen to a Fab' fragment from a monoclonal antibody that specifically recognizes a metal chelate (*see*, page 6651, col. 2, lines 8-14). There is

no disclosure in Stickney *et al.* of generating a mutant antibody by introducing a substitution, addition, or deletion in the nucleotide sequence encoding the antibody.

Therefore, Stickney *et al.* does not describe every element of the claimed mutant antibodies.

6. *The claimed compositions are not obvious over the disclosures of Reardan et al., Orlandi et al., Pastan et al., and Goodwin et al.*

The Examiner has alleged that the claimed compositions are obvious in view of the combination of the disclosures of Reardan *et al.*, Orlandi *et al.*, Pastan *et al.*, and Goodwin *et al.* In particular, the Examiner alleges that Pastan *et al.* teach a disulfide stabilized antibody comprising and SH group not present in the wild type antibody, and that Goodwin *et al.* teach a chelate comprising a reactive group of complementary reactivity to a reactive site.

a. The cited references fail to disclose each element of the presently claimed mutant antibodies and do not provide any motivation for one of skill in the art to develop the claimed mutant antibodies

i. Reardan et al.

I am the corresponding author of Reardan *et al.* and the experiments described therein were all conducted under my supervision. Reardan *et al.* describe a ***wild type*** monoclonal antibody, CHA255, which specifically recognizes a metal chelate. Reardan *et al.* do not disclose or suggest generating mutants of CHA255, or indeed, any mutant antibodies. Therefore, Reardan *et al.* do not provide any motivation for one of skill in the art to generate the claimed mutant antibodies that comprise a reactive site that interacts with a reactive group on a metal chelate.

ii. Orlandi et al.

Orlandi *et al.* describe cloning of the ***wild type*** of the variable regions of immunoglobulin molecules. Orlandi *et al.* does not disclose or suggest mutant antibodies. In fact, Orlandi *et al.* explicitly describes experiments to confirm that the sequences of the variable regions are accurately amplified (*see, e.g.*, page 3835, col. 2, lines 25-28). Thus, Orlandi *et al.*, provide no motivation for one of skill in the art to generate any mutant antibodies, much less

mutant antibodies which comprise a reactive site that interacts with a reactive group on a metal chelate.

iii. Pastan et al.

Pastan *et al.* describe a polypeptide comprising two separate variable regions of a ligand binding moiety connected via a disulfide bond (*see, e.g.*, col. 1, lines 61-66). Pastan *et al.* does not disclose or suggest that a reactive site on the mutant antibody may be in a location that would allow the site to react with a reactive group on the metal chelate. The disclosure of Pastan *et al.* does not even contain the term "metal chelate." Therefore, Pastan *et al.*, provide no motivation for one of skill in the art to generate mutant antibodies which comprise a reactive site that interacts with a reactive group on a metal chelate.

iv. Goodwin et al.

I am an author of Goodwin *et al.* and the majority of the experiments described therein were conducted with my collaboration. Goodwin *et al.* describe ***wild type*** monoclonal antibodies that specifically recognize and bind a 1, 4 dithiol spacer group on a metal chelate. The wild type antibodies of Goodwin *et al.* recognize and specifically bind to the spacer group itself. In contrast to the presently claimed mutant antibodies, the wild type antibodies of Goodwin *et al.*, do not comprise any mutations, much less mutations that are reactive sites with a complementary reactivity to a reactive group on a metal chelate. Therefore, Goodwin *et al.* do not provide any motivation for one of skill in the art to generate the claimed mutant antibodies that comprise a reactive site that interacts with a reactive group on a metal chelate.

Figure 1 of Goodwin *et al.* sets forth the structures of several metal chelates discussed in the references, *i.e.*, bromoacetamidobenzyl-EDTA (BABE) (Fig 1A); BABE conjugated to a protein via an iminothiolane spacer (Fig. 1B); and metal chelates comprising a 1,4-dithiol spacer (Figs. 1C and 1D). None of the compounds shown in Figure 1 comprise a reactive functional group of complementary reactivity to a reactive site on the wild type monoclonal antibodies described in Goodwin *et al.*

Therefore, Goodwin *et al.* does not provide any motivation for one of skill in the art to generate mutant antibodies which comprise a reactive site that interacts with a reactive group on a metal chelate.

Thus, ***none*** of the cited references disclose all of the elements of the claimed mutant antibodies which comprise a reactive site that interact with a reactive group on a metal chelate. Moreover, ***none*** of the cited references provide any motivation for a skilled artisan to generate such mutant antibodies.

b. Even if the cited references were combined, the combination would not lead to the presently claimed mutant antibody

As explained in detail above, none of the references disclose each element of the claimed mutant antibodies which comprise a reactive site that interacts with a reactive group on a metal chelate. Likewise, the combination of references does not disclose each element of the presently claimed antibody. In addition, the references alone or in combination not provide motivation for one of skill in the art to generate the presently claimed antibody. Three of the references: Reardan *et al.*, Orlandi *et al.*, and Goodwin *et al.* disclose only ***wild-type*** antibodies. There is no disclosure of suggestion in any of these references of any mutant antibody, much less a mutant antibody with mutation that is a reactive site with complementary reactivity to a reactive group on a metal chelate. One reference, Pastan *et al.* describes addition of a disulfide linkage between two separate variable regions of a polypeptide which is a ligand binding moiety (*e.g.*, an antibody) to ***stabilize*** the polypeptide. There is no disclosure or suggestion in Pastan *et al.* that the disulfide linkage is positioned in a location where it may interact with a reactive group on a metal chelate that is bound to an antibody. Moreover, Pastan *et al.* does not describe any metal chelates. Therefore, even if the cited references were combined, the combination would not lead to the presently claimed mutant antibody which comprise a reactive site that interacts with a reactive group on a metal chelate.

c. One of skill in the art would have no reasonable expectation of success in producing the claimed mutant antibody by combining the cited references

One of skill in the art would have no reasonable expectation of success in modifying the disclosures of the cited references to produce the claimed mutant antibodies comprising a reactive site that interacts with a reactive group on a metal chelate. As explained above, none of the cited references disclose or suggest that a reactive group on a mutant antibody may be placed in a location that would allow the reactive site on the antibody to react with a reactive group on a metal chelate bound by the antibody. Without the explicit guidance in the

specification of the present application regarding the placement of a reactive site on a mutant antibody having a reactive site not present on the wild-type antibody, wherein the reactive site is the mutation and interacts with a reactive group on the metal chelate (*see, e.g.*, page 71, line 1 to page 73, line 2). More particularly, the specification at page 71, lines 16-23 and page 73, lines 25-28 describes the use of computer aided design to select serine 95 as a suitable position for placement of a reactive site. Without these teachings, one of skill in the art would not have expected that modifying the cited references would successfully produce such an antibody.

7. *The claimed compositions are enabled by the specification as filed.*

It is my understanding that the Examiner is concerned that the specification does not enable a mutant antibody comprising any reactive site or a mutant antibody comprising a reactive site within the CDR. In particular, the Examiner alleges that the specification enables only mutant antibodies comprising a reactive site that is the SH group of cysteine, wherein the reactive site is proximate to a CDR.

a. The specification provides ample guidance, including actual working examples, for one of skill in the art to practice the full scope of the claimed invention

The specification provides ample guidance for one of skill in the art to practice the claimed invention, *i.e.*, mutant antibodies that recognize a metal chelate and comprise a reactive site not present on the wild-type of the antibody, wherein the reactive site interacts with a reactive group on the metal chelate. For example, the specification describes methods of generating mutant antibodies, including mutant antibodies that recognize a metal chelate (*see, e.g.*, page 23, line 20 to page 50, line 9); multiple types of reactive sites that can be introduced into the mutant antibodies (*see, e.g.*, page 47 line 16 to page 48, line 26); and multiple metal chelates with suitable reactive groups of complementary reactivity to the reactive site on the mutant antibodies (*see, e.g.*, page 61, line 25 to page 64, line 24).

The specification also provides working examples of (1) synthesis of an exemplary metal chelate with a reactive group (*see, e.g.*, page 67, line 19 to page 69, line 16); (2) generation of a mutant antibody comprising a reactive site that is the mutation, wherein the reactive site binds to a reactive group on a metal chelate (*see, e.g.*, page 71, line 1 to page 73, line 2); and (3) irreversible binding of an exemplary mutant antibody to a metal chelate with a

reactive group of complementary reactivity to the reactive site on the antibody (*see*, *e.g.*, page 73, line 4 to page 75, line 5).

b. *Based on the guidance in the specification and the working examples, one of skill in the art would appreciate that mutant antibodies comprising the claimed reactive sites are fully enabled*

As explained above, the specification describes multiple methods of generating mutant antibodies using methods well known in the art and include, for example, site directed mutagenesis, PCR mutagenesis, and cassette mutagenesis (*see*, *e.g.*, page 23, line 20 to page 31, line 14). In addition, the specification sets forth methods of expressing and purifying the mutant antibodies (*see*, *e.g.*, page 31, line 15 to page 46, line 28). Moreover, the specification sets forth multiple types of reactive sites that can be introduced into the mutant antibodies including cysteinyl residues, histidyl residues, lysinyl and other amino terminal residues, arginyl residues, tyrosyl residues, aspartyl residues, glutamyl residues, glutaminyl residues, asparaginyl residues, proline residues, and lysine residues (*see*, *e.g.*, page 52 line 16 to page 55, line 2). Thus, there is ample guidance in the specification for one of skill in the art to practice the full scope of the claimed invention.

In addition to the guidance in the specification regarding generation of a mutant antibody, the specification provides two working examples which demonstrate: (1) generation of a mutant antibody comprising a reactive site that is the mutation, wherein the reactive site binds to a reactive group on a metal chelate (*see*, *e.g.*, page 71, line 1 to page 73, line 2); and (2) irreversible binding of a mutant antibody to a metal chelate with a reactive group of complementary reactivity to the reactive site on the antibody.

More particularly, Example 3 (page 71, line 1 to page 73, line 2) describes generation of a mutant CHA255 antibody comprising a reactive site not present on the wild type CHA255 antibody using computer aided design to identify suitable placement of a mutation, *i.e.*, substitution of a native amino acid residue for a reactive site. As set forth in Example 3, a serine residue at position 95 (S95) of the light chain of CHA 255 and an asparagine residue at position 96 (N96) of the light chain of CHA 255 were chosen for substitution because of their proximity to the para-substituent acryl group of a metal chelate (*i.e.*, (S)-*p*-acrylaminobenzyl-EDTA-In chelate) when the metal chelate is bound to the CHA255. The serine and asparagine was also

chosen for substitution because it was identified as a residue that was not involved in the van der Waals interactions between CHA255 and the metal chelate. Example 3 further sets forth mutagenesis of the S95 to cysteine and the N96 to cysteine to generate mutant CHA255 light chains and expression of the mutant CHA255 light chains in *Drosophila* cells.

Example 4 (page 73, line 5 to page 75, line 5) describes binding of a mutant CHA255 antibody comprising a reactive site not present on the wild type CHA255 to a metal chelate with complementary reactivity to the reactive site on the mutant CHA255 antibody. More particularly, Example 4 describes binding of the mutant antibodies of Example 3 to ^{111}In-labeled metal chelates bearing acrylamido, chloropropionamido, or chloroacetamido groups.

Thus, based on the guidance in the specification and the teachings of the actual working Examples 3 and 4, one of skill in the art would appreciate that the claimed mutant antibodies are fully enabled.

*c. Based on the guidance in the specification and the working examples, one of skill in the art would appreciate that mutant antibodies comprising a reactive site not present in the wild type of the antibody, wherein the reactive site is proximate to **or** within the CDR are fully enabled*

As explained above, the specification describes multiple methods of generating mutant antibodies using methods well known in the art and include, for example, site directed mutagenesis, PCR mutagenesis, and cassette mutagenesis (*see*, *e.g.*, page 23, line 20 to page 31, line 14). In addition, the specification sets forth methods of expressing and purifying the mutant antibodies (*see*, *e.g.*, page 31, line 15 to page 46, line 28). Moreover, the specification sets forth multiple types of reactive sites that can be introduced into the mutant antibodies, including, for example, cysteinyl residues, histidyl residues, lysinyl and other amino terminal residues, arginyl residues, tyrosyl residues, aspartyl residues, glutamyl residues, glutaminyl residues, asparaginyl residues, proline residues, and lysine residues (*see*, *e.g.*, page 52 line 16 to page 55, line 2). Thus, there is ample guidance in the specification for one of skill in the art to practice the full scope of the claimed invention.

In addition to the guidance in the specification regarding generation of a mutant antibody, the specification provides a working example which demonstrates: generation of a mutant antibody comprising a reactive site that is the mutation, wherein the reactive site binds to

a reactive group on a metal chelate (*see, e.g.*, page 71, line 1 to page 73, line 2) and wherein the reactive site is within the CDR of the antibody.

More particularly, Example 3 (page 71, line 1 to page 73, line 2) describes generation of a mutant CHA255 antibody comprising a reactive site not present on the wild type CHA255 antibody using computer aided design to identify ***suitable placement of a mutation***, *e.g.*, a reactive site. As set forth in Example 3, a serine residue at position 95 (S95) of the light chain of CHA255 and an asparagine residue at position 96 (N96) of the light chain of CHA255 were chosen for substitution because of their proximity to the acryl group of a metal chelate (*i.e.*, (S)-*p*-acrylaminobenzyl-EDTA-In chelate) when the metal chelate is bound to the CHA255. The serine and asparagine was also chosen for substitution because it was identified as a residue that was not involved in the hydrophobic interactions or hydrogen bonding between CHA255 and the metal chelate. Each of the substituted residues is within the CDR of CHA255. Example 3 further sets forth mutagenesis of the S95 to cysteine and the N96 to cysteine to generate a mutant CHA255 light chain, and expression of the mutant CHA255 light chain in *Drosophila* cells.

Thus, as set forth in Example 3, using methods known in the art, a skilled artisan would be able to identify suitable positions for placement of a mutation in an antibody. In particular, one of skill in the art would be able to identify suitable positions proximate to or within the CDR of an antibody which can be substituted for a reactive site, *i.e.*, a reactive site that has complementary reactivity to a reactive group on a metal chelate.

Accordingly, based on the guidance in the specification and the disclosure of working Example 3, one of skill in the art would appreciate that the claimed mutant antibodies are fully enabled.

8. In view of the foregoing, it is my scientific opinion that the claimed mutant antibodies are novel and not obvious in view of the cited art. Moreover, it is my scientific opinion that the claimed mutant antibodies are fully enabled by the specification as filed.

Date: Feb 24, 2004 By: Claude Meares

Claude Meares, Ph.D.

BIOGRAPHICAL SKETCH

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Born: September 25, 1946, in Wilmington, North Carolina.

Education

B. S. in Chemistry
University of North Carolina, Chapel Hill,
North Carolina, 1968 (awarded with highest honors)

Ph. D. in Chemistry
Stanford University, Stanford, California, 1972.

Employment

Chemistry Faculty, University of California, Davis

Assistant Professor	1972-1978
Associate Professor	1978-1982
Professor	1982-present
Dept. Chair	1997-2000

Honors

Association for Laboratory Automation Achievement Award, 2002
Immunomedics Science Award, 1998
Fellow, American Association for the Advancement of Science, 1994
Distinguished Scientist Award, 1994, Society of Nuclear Medicine, Western Region
Visiting Committee, Brookhaven National Laboratory Medical Department, 1993-1997
Editor-In-Chief, ***Bioconjugate Chemistry***, 1989- (American Chemical Society)
Member, Board of Editors, ***Inorganic Chemistry***, 1989- (American Chemical Society)
NIH MERIT award, 1991 (CA 16861)
Member, Metallobiochemistry Study Section, NIH Division of Research Grants, 1982-1986
NIH Research Career Development Award, 1979-1984
von Hevesy Prize for Nuclear Medicine, 1974
NSF Graduate Fellow, 1968-1972, Stanford University
Venable Medal, 1968, University of North Carolina
Phi Beta Kappa, 1967, University of North Carolina

Research Interests

Bioconjugate chemistry, molecular biology, application of chemical techniques to biological and biomedical problems, mapping protein-protein and protein-nucleic acid interactions, bifunctional chelating agents, engineered antibodies.

BIOGRAPHICAL SKETCH

Publications
See attached list.

1. Reuben D. Rieke, Claude F. Meares, and Loretta I. Rieke. Ring Strain Effects on Spin Densities — 1. Ring Strain Effects on Spin Densities in Substituted Naphthalene Radical Anions. *Tetrahedron Letters* **51**, 5275-5278 (1968).
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8. David A. Goodwin, Claude F. Meares, and Chung H. Song. Study of 111 Indium-Labeled Compounds in Mice, using Perturbed Angular Correlations of Gamma Radiations. *Radiology* **105**, 699-702 (1972).
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13. David A. Goodwin, Michael W. Sundberg, Carol I. Diamanti, and Claude F. Meares. Indium-111 Radiopharmaceuticals in Cancer Localization. *Tumor Diagnosis (Radiologic and other Biophysical Methods)* , 57-88 (1975).
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17. Claude F. Meares, David A. Goodwin, Charles S-H. Leung, Amal Y. Girgis, David J. Silvester, Adrian D. Nunn, and Peter J. Lavender. Covalent Attachment of Metal Chelates to Proteins: The Stability in Vivo and in Vitro of the Conjugate of Albumin with a Chelate of 111-Indium. *Proceedings of the National Academy of Sciences (USA)* **73**, 3803-3806 (1976).

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